Ant species identity mediates reproductive traits and allocation in an ant-garden bromeliad

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• *Background and Aims* Determining the sources of variation in floral morphology is crucial to understanding the mechanisms underlying Angiosperm evolution. The selection of floral and reproductive traits is influenced by the plant's abiotic environment, florivores and pollinators. However, evidence that variations in floral traits result from mutualistic interactions with insects other than pollinators is lacking in the published literature and has rarely been investigated. We aimed to determine whether the association with either *Camponotus femoratus* or *Pachycondyla goeldii* (both involved in seed dispersal and plant protection) mediates the reproductive traits and allocation of *Aechmea mertensii*, an obligatory ant-garden tank-bromeliad, differently.

• *Methods* Floral and reproductive traits were compared between the two *A. mertensii* ant-gardens. The nitrogen flux from the ants to the bromeliads was investigated through experimental enrichments with stable isotopes (^{15}N).

• *Key Results Camponotus femoratus*-associated bromeliads produced inflorescences up to four times longer than did *P. goeldii*-associated bromeliads. Also, the numbers of flowers and fruits were close to four times higher, and the number of seeds and their mass per fruit were close to 1·5 times higher in *C. femoratus* than in *P. goeldii*-associated bromeliads. Furthermore, the ^{15}N-enrichment experiment showed that *C. femoratus*-associated bromeliads received more nitrogen from ants than did *P. goeldii*-associated bromeliads, with subsequent positive repercussions on floral development. Greater benefits were conferred to *A. mertensii* by the association with *C. femoratus* compared with *P. goeldii* ants.

• *Conclusions* We show for the first time that mutualistic associations with ants can result in an enhanced reproductive allocation for the bromeliad *A. mertensii*. Nevertheless, the strength and direction of the selection of floral and fruit traits change based on the ant species and were not related to light exposure. The different activities and ecological preferences of the ants may play a contrasting role in shaping plant evolution and speciation.

Key words: *Aechmea mertensii*, *Camponotus femoratus*, bromeliad, Bromeliaceae, $\delta^{15}N$, floral traits, fruit-set, mutualistic ants, *Pachycondyla goeldii*, reproductive allocation, stable isotopes.

INTRODUCTION

Floral traits play an important role in the dynamics of plant populations, primarily because their variations affect the attractiveness of flowers to pollinators and can subsequently influence plant fitness (Strauss *et al.*, 1996; Mothershead and Marquis, 2000). Determining the sources of variation in floral morphology is therefore of crucial importance to broadening our understanding of the mechanisms underlying Angiosperm evolution. For a given plant species, the variations in floral traits result from pluralistic processes and causes (Galen, 1999). The proximate causes of phenotypic plasticity in plants concern the physical environment (inter alia: incident light, temperature, nutrient intake and elevation) (Frazee and Marquis, 1994; Strauss and Whittall, 2006). Ultimately, the process of diversification in floral traits must be carried out by pollinators (Fenster *et al.*, 2004; Parachnowitsch and Kessler, 2010) and florivores (Cascante-Marin *et al.*, 2009; Hanley *et al.*, 2009). Variations in floral traits that increase plant fitness and involve mutualistic insects other than pollinators have not been reported to the best of our knowledge.

Ants are amongst the most abundant and ecologically important arthropods in tropical rain forests, accounting for 20–40 % of the arthropod biomass and up to one-third of all of the mutualisms between arthropods and woody plant species (Beattie, 1985). Ant–plant relationships range from simple opportunism and mutual benefit to complex, multiple interactions (Vazquez *et al.*, 2009). Numerous studies have shown that ants play a major role in (1) seed dispersal (Howe and Smallwood, 1982; Brew *et al.*, 1989), (2) the protection of leaves from herbivory (Fonseca, 1994; Heil and McKey, 2003), (3) macronutrient supply (Treseder *et al.*,

1995; Fischer *et al.*, 2003), (4) defending the plant's reproductive organs (Horvitz and Schemske, 1984; Vesprini *et al.*, 2003), and, in some rare cases, (5) pollination (de Vega *et al.*, 2009). Thus, an association with ants should a priori result in a higher plant reproductive output (Gaume *et al.*, 2005*b*; but see Letourneau, 1998). Nevertheless, if ants can increase plant fitness by deterring phytophagous insects and by disseminating seeds, they may also impose reproductive costs on their host plants. Such costs are the result of either their predatory behaviour towards effective pollinators or damage caused directly to the reproductive parts of the plants (Yu and Pierce, 1998; Izzo and Vasconcelos, 2002; Gaume *et al.*, 2005*a*; Ness, 2006; Frederickson, 2009; Orivel *et al.*, 2011). Also, the sap-sucking Hemiptera exploited and disseminated by ants may dramatically affect flower structures and the plant's reproductive biology (Ivey and Carr, 2005). In obligate ant–plant interactions, the identity of the mutualistic ant species is therefore an important factor influencing (positively or negatively) plant fitness.

Among tropical plants, epiphytes represent a keystone resource in rain forests because of their important role in nutrient cycling and in providing habitats for many microorganisms, invertebrates and small vertebrates (Nadkarni, 1994). Some epiphytic species have developed symbioses with ants, either by providing chambers (domatia) where ants nest (Davidson and Epstein, 1989) or by rooting in arboreal ant gardens (AGs) (Benzing, 2000; Orivel and Leroy, 2011). AGs are initiated by a few ant species whose founding queens and/or workers build arboreal carton nests. The main benefits for the plant combine the principal positive outcomes from both seed dispersal and protective mutualisms (Orivel and Leroy, 2011). The ants collect and incorporate the seeds of selected epiphyte species which then germinate and grow on the nest, so that the plant roots stabilize and anchor the entire structure to the supporting tree (Orivel *et al.*, 1998).

Over its entire (South American) range, the tank-bromeliad *Aechmea mertensii* occurs only in association with AGs (Benzing, 2000). In French Guiana, *A. mertensii* is found in secondary forest formations (pioneer growths) on AGs initiated either by *Camponotus femoratus* or by *Pachycondyla goeldii* ants (Corbara and Dejean, 1996; Vantaux *et al.*, 2007). As dispersal and protective agents for this bromeliad, *C. femoratus* and *P. goeldii* indirectly influence its vegetative traits (i.e. plant shape and size, leaf anatomy) by determining the location of the seedling, from exposed to partially shaded areas, respectively (Leroy *et al.*, 2009*a*). This recent study showed that variation in some vegetative traits (i.e. the size and shape of the bromeliad) were related to a light acclimation process whereas others (i.e. leaf thickness and leaf mass per unit area) were related to nutrient-stressed environments linked to the identity of the associated ant. Despite this variation in plant forms and vegetative traits, it was ascertained that both *C. femoratus*- and *P. goeldii*-associated bromeliads belong to the same species (Céréghino *et al.*, 2011).

In the present study, we investigated the influence of its two mutualistic ant species, *C. femoratus* and *P. goeldii*, on the reproductive allocation of *A. mertensii*. Assuming that associations with ants having different ecological requirements affect the outcome of the mutualism for the plant (Leroy *et al.*, 2009*a*; Céréghino *et al.*, 2010), we hypothesized that the plant's reproductive traits (i.e. its flowers and seeds) depend more on the species of associated ant rather than on exposure to light. Furthermore, as $\delta^{15}N$ values can be used as indicators of the nitrogen source (Leroy *et al.*, 2009*b*), an experiment using ^{15}N-enriched food provided to the ants was also carried out to investigate if ant-foraged nitrogen can enhance the plant's reproductive traits and allocation.

MATERIALS AND METHODS

Study site and species characteristics

This study was conducted from October 2008 to January 2009 and from October to November 2009 in pioneer growths along forest edges around the field station at Petit-Saut, Sinnamary, French Guiana (05°03′30·0″N, 52°58′34·6″W; elevation 100 m a.s.l.). The climate is tropical moist, with 3500 mm of yearly precipitation distributed over 280 d. A major drop in rainfall occurs between July and November (dry season), and another shorter, more irregular dry period occurs in March. The maximum monthly temperature averages around 33·5 °C, and the monthly minimum around 20·3 °C. The percentages of total incident light received by each of the plants studied were estimated using hemispherical photography (for a more detailed methodology, see Leroy *et al.*, 2009*a*).

All of the plants studied were located adjacent to a dirt road on well-developed and easily accessible AGs inhabited by the ants *Camponotus femoratus* Fabr. and *Crematogaster levior* Longino or by *Pachycondyla goeldii* Forel. *Camponotus femoratus* is a polygynous (multiple queens), arboreal formicine species living in a parabiotic association with the myrmicine species *Cr. levior*; that is to say, they share the same nests and trails, but shelter in different cavities of the nests (Orivel *et al.*, 1997; Vantaux *et al.*, 2007). Their large polydomous (multiple nests) colonies and aggressiveness identify them as territorially dominant species in Neotropical rain forest canopies. *Pachycondyla goeldii*, by contrast, is a monogynous (single queen) arboreal ponerine species with smaller populations, although the colonies may be polydomous (Corbara and Dejean, 1996; Dejean *et al.*, 2000).

Aechmea mertensii Schult.f. (Bromeliaceae) (subfamily Bromelioideae) has tightly interlocking leaves forming compartments that collect water and organic detritus. These tanks, or phytotelmata ('plant-held water'), provide a habitat for aquatic micro- and macro-organisms as well as for vertebrates (Richardson, 1999; Carrias *et al.*, 2001; Brouard *et al.*, 2011). *Aechmea mertensii* is characterized by sympodial branching that leads to a series of attached, compact, terminally flowered ramets (Benzing, 2000). Inflorescences are supported by a long reddish brown peduncle projecting the inflorescence above the rosette (Mori *et al.*, 1997; Fig. 1). The peduncle has spirally arranged red to pink bracts (Fig. 1A, B). The inflorescences, one-branched, are composed of spikes with 4–12 flowers. The flowers are hermaphroditic and actinomorphic with fleshy yellow calyces composed of three sepals and red corollas composed of three petals. Stamens arise in two whorls of three members each, and are attached to the corolla. The gynoecium consists of three carpels with inferior ovaries. A septal nectary is present in

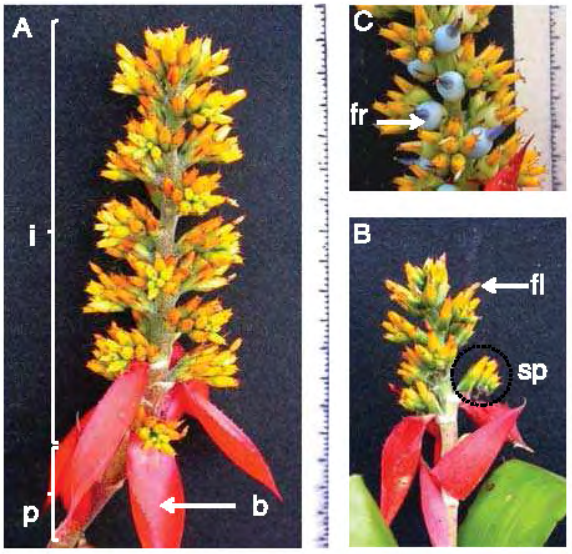

FIG. 1. Morphology of *Aechmea mertensii* inflorescences on (A) *Camponotus femoratus*-associated bromeliads, and (B) *Pachycondyla goeldii*-associated bromeliads. Appearance of the first blue-coloured berries on a *Camponotus femoratus*-associated bromeliad with (C) extremity of an inflorescence with ripe fruits. Abbreviations: b = bract of the peduncle, fl = flower, fr = fruit, i = inflorescence, p = peduncle, sp = spike. Scale divisions in millimetres.

the interlocular position. The fruits are blue, spine-armed berries containing naked seeds with appendages (Fig. 1C).

Floral trait measurements

Floral and fruit characteristics were measured during the reproductive period (October 2008 to January 2009) for *C. femoratus*- and *P. goeldii*-associated plants ($n = 22$ and 26 *A. mertensii*, respectively). We measured the diameter (two random measurements taken at 90°) of the reservoir and the height of the inflorescence, and recorded the number of spikes and flowers per inflorescence (Fig. 1A, B). We collected five flowers from the centre of the inflorescence for each of the plants studied (110 and 130 flowers from *C. femoratus*- and *P. goeldii*-associated plants, respectively). Flower length and width were measured using a stereomicroscope equipped with a micrometer.

Pollen/ovule ratio

The flowers examined for pollen/ovule ratios (P/O) were near anthesis; thus the pollen was mature, but the anthers had not dehisced. To estimate the number of pollen grains per stamen, we collected three stamens each with one anther per flower. Each anther was digested in 300 μL of 95 % sulphuric acid for 2 d at 24 °C. The solution was then homogenized, and 1 μL was collected and carefully placed on a microscope slide. The number of pollen grains (N) was counted for five independent replicates of 1 μL. The total number of pollen grains per stamen was obtained by multiplying the mean of the five replicate totals by 300 and multiplying the result by the average number of stamens (n). Thus, the P/O ratio used is $300 \times N \times n$ divided by the number of ovules in the ovary (Cruden, 1977).

Fruit- and seed-set

We periodically monitored fruit development until maturation. After counting the number of flowers, and then mature fruits, the evaluation of fruiting success was based on the 'fruit-set'; i.e. the percentage of flowers developing into a mature fruit (Burne *et al.*, 2003). For each of the plants studied, five ripe (blue-tinged) fruits were collected from the centre of the inflorescence (110 and 130 fruits from *C. femoratus*- and *P. goeldii*-associated plants, respectively). Using a stereomicroscope equipped with a micrometer, we measured the length and width of the fruits, and counted the number of seeds per fruit. We then distinguished mature (i.e. well-developed) seeds from aborted or unfertilized seeds (i.e. ovules that failed to form seeds). All of the mature seeds in each fruit were then dried and weighed using a quartz crystal microbalance.

^{15}N enrichment

We investigated the role of the two mutualistic ant species in provisioning *A. mertensii* with nitrogen at the flowering stage by providing colonies with food artificially enriched with ^{15}N. Between October and November 2009, we monitored 18 and 12 *C. femoratus*- and *P. goeldii*-associated plants, respectively, providing colonies *ad libitum* with food artificially enriched with ^{15}N every 2 d for 3 weeks. The quantity of artificial food provided each time depended on the size of the AGs, itself related to the size of the colonies and the number of epiphytic plant species (*C. femoratus*-AGs are three to four times larger and host a more populous ant colony and number of plant species than *P. goeldii*-AGs). A preliminary study conducted using non-enriched foods permitted us to obtain (1) the preferred artificial food for both ant species, and (2) an approximate idea of the amount of food consumed by each ant colony. This varied from 6 to 12 g for *C. femoratus* colonies, and from 4 to 6 g for *P. goeldii* colonies. We then provided these colonies with the corresponding quantities of food artificially enriched with ^{15}N. The food was placed in small plastic cups, covered to keep out food-robbing insects, attached to a branch on the host tree 20–40 cm away from the AGs. No physical contact occurred between these cups and the epiphytic plants or the carton nests. Before re-supplying each colony with a new, predefined quantity of fresh food artificially enriched with ^{15}N, we cleaned the cups, removing any remaining food.

The food was artificially enriched with ^{15}N as follows. We first boiled 375 mL of distilled water with 30 g Agar-Agar (Sigma, St Louis, MO, USA). Then, 750 g of mealworms was mixed into another 375 g of distilled water containing 3 g methyl 4-hydroxybenzoate (an antifungal agent used as a food preservative) plus 10 g ammonium nitrate ($NH_4^{15}NO_3$, 10 at.% ^{15}N, Isotec, Sigma-Aldrich.com/isotec), 10 g ammonium nitrate ($^{15}NH_4NO_3$, 10 at.% ^{15}N, Isotec) and 10 g urea ($H_2^{15}NCO^{15}NH_2$, 10 at.% ^{15}N, Isotec). Finally, this preparation was mixed with the agar solution. This artificially enriched food was kept in a refrigerator at 4 °C during the entire experimental period.

Isotopic analysis

Pieces of leaves and flowers were collected before ^{15}N enrichment and 1 week after the 3-week-long ^{15}N enrichment experiment. All of the samples were vacuum-dried and ground into a homogeneous powder using a mixer mill. Around 1 g from each plant sampled was analysed for its total N and $\delta^{15}N$ content. Stable isotope analyses were conducted at the Colorado Plateau Stable Isotope Laboratory (Northern Arizona University, USA) using a Thermo-Finnigan Delta^{plus} Advantage gas isotope-ratio mass spectrometer interfaced with a Costech Analytical ECS4010 elemental analyser. The natural abundances of ^{15}N were calculated as follows:

$$\delta X = (R_{sample}/R_{standard} - 1) \times 1 \cdot 000$$

where X is the element of interest, and R_{sample} and $R_{standard}$ are the molar ratios (i.e.$^{15}N/^{14}N$) of the sample and the standard, respectively (DeNiro and Epstein, 1978).

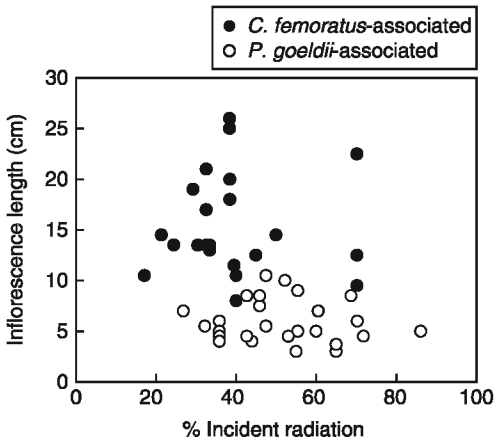

FIG. 2. Relationship between the length of the bromeliad *A. mertensii* inflorescences and the light environment (% incident radiation) in relation to the distribution of its ant partner: *Camponotus femoratus*-associated bromeliads ($n = 22$), *Pachycondyla goeldii*-associated bromeliads ($n = 26$), as indicated.

Statistical analyses

Preliminary tests showed that most of the variables were not normally distributed (Shapiro Wilk's test) even after transformation. Thus, Mann–Whitney *U*-tests (Statistica 8 software; Statsoft Inc, Tulsa, OK, USA) were used to test significant differences in incident light; inflorescence, and floral and fruit traits; and total leaf and flower N and $\delta^{15}N$ based on ant species. Finally, a Wilcoxon matched-pairs test was used to compare the differential $\delta^{15}N$ enrichment of the leaves and flowers.

RESULTS

Incident light

Camponotus femoratus-AGs received significantly less transmitted light than *Pachycondyla goeldii*-AGs (mean ± s.d. 38·57 ± 5·19 vs. 52·64 ± 5·34 %, respectively, $U = -3{\cdot}55$, $P < 0{\cdot}0001$). However, the distribution of the two AG-ant species showed a gradient from *C. femoratus*-AGs to *P. goeldii*-AGs with a clear overlapping of the incident light for the two ant species (Fig. 2). If we plot the length of *A. mertensii* inflorescences against incident light, it clearly appears that no relationship exists between these two variables either for *C. femoratus*-associated bromeliads ($R^2 = 0{\cdot}0006$, $P = 0{\cdot}99$) or for *P. goeldii*-associated bromeliads ($R^2 = 0{\cdot}027$, $P = 0{\cdot}40$).

Floral features

Compared with *P. goeldii*-associated plants, *C. femoratus*-associated plants had inflorescences three to four times longer, with up to four times more spikes and flowers per inflorescence, longer and wider flowers, and a higher number of ovules and pollen grains per flower (Table 1; see also Fig. 1). In both cases, the length of the inflorescences and the number of flowers per inflorescence showed a significant positive relationship with reservoir size (Fig. 3A, B). More interestingly, for a similar reservoir size, inflorescence length and the number of flowers per inflorescence were significantly higher for *C. femoratus*-associated bromeliads (Fig. 3C).

TABLE 1. *Inflorescence, flower and fruit traits of* Aechmea mertensii *associated with either* Camponotus femoratus *(*C.f*-bromeliads,* n = *22) or* Pachycondyla goeldii *(*P.g*-bromeliads,* n = *26)*

	C.f-bromeliads	*P.g*-bromeliads	*U*-test	*P*
Inflorescence traits				
Inflorescence length (cm)	18·21 ± 2·03	5·71 ± 1·08	5·92	<0·0001
No. of spikes per inflorescence	26·95 ± 4·10	10·11 ± 2·14	5·79	<0·0001
No. of flowers per inflorescence	256·61 ± 61·55	64·24 ± 17·22	5·79	<0·0001
Flower traits				
Flower length (mm)	1·30 ± 0·04	0·99 ± 0·04	5·92	<0·0001
Flower width (mm)	0·36 ± 0·01	0·32 ± 0·01	5·52	<0·0001
No. of ovules per flower	12·67 ± 0·53	9·29 ± 0·43	5·91	<0·0001
No. of pollen grains per flower	5825 ± 1985·85	3238 ± 1103·41	3·42	<0·0005
P/O ratio	479·25 ± 93·71	352·02 ± 60·96	1·92	0·054
Fruit traits				
No. of fruits per inflorescence	209·52 ± 45·21	56·18 ± 12·06	5·66	<0·0001
Fruit-set (%)	84·43 ± 4·84	85·32 ± 2·88	0·55	0·577
No. of seeds per fruit	10·71 ± 0·89	7·50 ± 0·93	3·99	<0·0001
Seed mass per fruit (mg)	2·67 ± 0·32	1·84 ± 0·24	3·41	<0·0001

Comparison of both *A. mertensii* ant-garden variables (mean ± s.d.) were conducted using the non-parametric Mann–Whitney *U*-test.

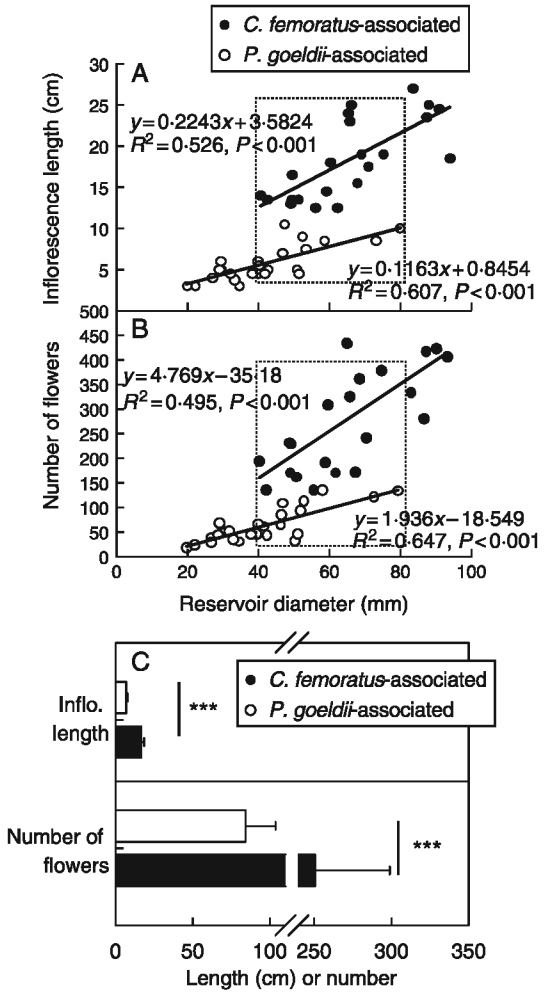

FIG. 3. (A) Inflorescence length and (B) number of flowers per inflorescence as a function of the diameter of the *A. mertensii* reservoir. *Camponotus femoratus*-associated bromeliads ($n = 22$); *Pachycondyla goeldii*-associated bromeliads ($n = 26$), as indicated. Dotted squares indicate *C. femoratus*- and *P. goeldii*-associated bromeliads with a similar range of tank diameters selected for (C). (C) Mean inflorescence length and number of flowers per inflorescence for *A. mertensii* with similar tank diameters on both *C. femoratus*- and *P. goeldii*-associated AGs (individuals inside the dotted squares in parts A, B): *C. femoratus*-associated bromeliads ($n = 17$), *P. goeldii*-associated bromeliads ($n = 14$), as indicated. Statistical analyses were conducted using the Mann–Whitney *U*-test. ***$P < 0.001$.

The P/O ratio, which was not significantly different between the two ant–bromeliad associations, ranged from 168 to 852. Based on Cruden's (1977) P/O categories, the breeding system used by *A. mertensii* ranges from facultative autogamous (i.e. P/O = 168·5 ± 22·1) to facultative allogamous (i.e. P/O = 796·6 ± 87·7), whereas obligate allogamous species are characterized by a higher P/O ratio (i.e. 5859·2 ± 936·5).

Fruit- and seed-set

The number of fruits per inflorescence was over three times higher for *C. femoratus*-associated plants than for *P. goeldii*-associated plants (Table 1). Fruit-set is very high

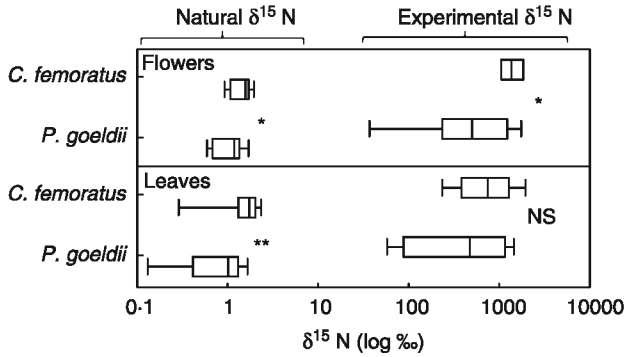

FIG. 4. Effect of ^{15}N provisioning of ants on *Aechmea mertensii* vegetative and floral parts. Natural and experimental $\delta^{15}N$ values for leaves ($n = 18$) and flowers ($n = 9$) on *Camponotus femoratus*-bromeliads and for leaves ($n = 12$) and flowers ($n = 9$) on *Pachycondyla goeldii*-bromeliads. Error bars indicate the 90th and 10th percentiles, the ends of the boxes indicate the 25th and 75th percentiles. Statistical analyses were conducted using the Mann–Whitney *U*-test: NS = no significant differences, *$P < 0.05$, **$P < 0.005$.

under natural conditions and was not significantly different in either ant–bromeliad association with more than 80 % of the flowers developing into a mature fruit. This high fruit-set associated with the observed P/O ratio indicates that *A. mertensii* spontaneously self-pollinates regardless of the ant partner. The number of seeds and the seed mass per fruit were significantly higher in *C. femoratus*-associated plants than in *P. goeldii*-associated plants (Table 1).

Tracing ant-foraged nitrogen through the addition of a ^{15}N tracer

Bromeliads associated with *C. femoratus* showed significantly higher total N and natural $\delta^{15}N$ values for leaf tissues than those associated with *P. goeldii* ($N = 0.61 \pm 0.06$ vs. 0.50 ± 0.06 %, $U = 2.271$, $P = 0.022$; $\delta^{15}N = 1.61 \pm 0.35$ vs. 0.88 ± 0.24 ‰, $U = 3.005$, $P = 0.002$). Provisioning the ants with ^{15}N-enriched food resulted in a more than 600 % increase in leaf and flower $\delta^{15}N$ (Fig. 4). The $\delta^{15}N$ in the leaves did not vary significantly between the plants based on the plant's ant mutualist ($\delta^{15}N = 824.09 \pm 279.60$ vs. 613.10 ± 307.29 ‰ for *C. femoratus*- and *P. goeldii*-associated bromeliads, respectively; $U = 1.481$, $P = 0.146$). However, flowers on *C. femoratus*-associated plants were significantly more enriched with ^{15}N than those on *P. goeldii*-associated plants ($\delta^{15}N = 1459.59 \pm 296.58$ vs. 732.97 ± 387.25 ‰; $U = 2.428$, $P = 0.014$). Moreover, $\delta^{15}N$ was significantly higher in flowers than in leaves at the end of the experiment for *C. femoratus*-associated plants (Wilcoxon matched-pairs test; $z = 2.665$, $P = 0.007$; Fig. 3); this was not the case for those bromeliads associated with *P. goeldii* ($z = 1.540$, $P = 0.123$).

DISCUSSION

In tandem with previous investigations, our results show that the two AG-ant species have a contrasting impact on the structural plasticity of *A. mertensii* for both vegetative (see Leroy *et al.*, 2009*a*) and floral traits (present study). While numerous studies have shown that the identity of the associated ant

species can differentially affect (1) plant protection (Gaume *et al.*, 2005*b*), (2) plant size and growth (Frederickson, 2005) and (3) reproductive allocation (Horvitz and Schemske, 1984; Vesprini *et al.*, 2003) or flower castration (Yu and Pierce, 1998; Gaume *et al.*, 2005*a*; Malé *et al.*, 2011; Orivel *et al.*, 2011), to the best of our knowledge, no previous study has demonstrated variations in floral and reproductive traits linked to the identity of the mutualistic ant species. Thus, the present study brings new insights into how mutualistic ants impact the floral traits of their host plant. The production of flowers, fruits and seeds by the bromeliad *A. mertensii*, as well as the structural characteristics of these organs, clearly depend on the species of its associated ant. Interestingly, while the incident light received by plants can explain phenotypic plasticity in the vegetative traits, we showed that the floral traits of *A. mertensii* were not primarily influenced by this factor. As association with AGs is obligatory for the bromeliad, our results suggest that greater benefits are conferred to this plant by the association with *C. femoratus* compared with the association with *P. goeldii*.

In epiphytic tank-bromeliads, the main source of nutrients comes from the phytotelmata formed by the tightly interlocking leaves which collect water, leaf litter and other organic detritus, and provide habitat for invertebrates (Benzing, 2000). By having larger phytotelmata, *C. femoratus*-associated plants host higher numbers of aquatic invertebrate species and individuals (Céréghino *et al.*, 2010), so that greater amounts of nitrogen from invertebrate faeces are made available to the bromeliad (Leroy *et al.*, 2009*a*). *Camponotus femoratus*-associated plants have the potential for greater nutrient allocation to inflorescences, flowers and seeds than do *P. goeldii*-associated plants, presumably due in part to the difference in the size of the phytotelmata. However, as we show here, with a similar phytotelm size, *C. femoratus*-associated plants produce more flowers and fruits per inflorescence than *P. goeldii*-associated plants. This observation suggests that ants indirectly influence *A. mertensii* nutrition via the phytotelm, but, on the other hand, it underlines the differential effect that the two main mutualistic ants have on the reproductive biology of the host plant. In the present study, we provide new evidence that ants could play a direct role in the transfer of nutrients to the plants probably through the plants' roots. Indeed, we found a significant increase in the relative abundance of the ^{15}N isotope in plant tissues after both ant species were supplied with ^{15}N-enriched food. Interestingly, *C. femoratus*-associated plants had the higher $\delta^{15}N$ values, indicating that members of this ant species might be better able to pass nitrogen to the host plant compared with *P. goeldii*. In addition, *C. femoratus*-associated plants allocated more resources to male (pollen grain number) and female (ovule number) functions compared with *P. goeldii*-associated plants. These results are in keeping with the resource–cost hypothesis postulated by Galen (1999) that predicts that reduced flower (inflorescence) size is advantageous under resource-poor conditions. Bromeliads may thus adjust to differences in resource availability through plastic changes in allocation to flowering.

According to Wyatt (1982), the function of the inflorescence in attracting insects is best fulfilled through large, showy floral displays, so that the inflorescences on *A. mertensii* associated with *C. femoratus* should be more attractive to diurnal pollinators than those on individuals associated with *P. goeldii*. Yet, during observations and measurements made on *A. mertensii* inflorescences throughout the day, no pollinating visitors were noted on the flowers on either kind of AG despite their bright red and yellow colours, so that we deduced that they are infrequent at least during daytime. Outcrossing (i.e. pollination by bats or nocturnal insects) cannot be ruled out as no observations were conducted at night, but it is likely that this species is mostly self-pollinated based on the P/O ratio and high fruit-set. In addition to a deficiency in pollinator services, several other selective factors may promote the evolution of self-pollination, such as the cost of outcrossing, low population density and selection for local adaptation (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987). For the majority of AG epiphytes, the high incidence of autogamy may be triggered by the aggressive behaviour of the ants toward pollinators (Madison, 1979). In *C. femoratus*-associated AGs, the ants are actively present on the inflorescence but never damage the flowers, while their presence and aggressive behaviour could deter both pollinators and flower and fruit feeders. Thus, the bright colour of *A. mertensii* inflorescences might be an ancestral trait with no or very little importance vis-à-vis pollinators (Saito and Harborne, 2001). These bright-coloured inflorescences (red bracts) and blue fruits probably rather play a role in seed dispersal by birds as they can be attracted by these colours (Stiles, 1976).

Based on our results, *C. femoratus* seems to be the best mutualistic ant partner for *A. mertensii* when compared with *P. goeldii*. As *C. femoratus*-associated bromeliads produce more seeds, natural selection should favour plants associated with *C. femoratus* more than plants associated with *P. goeldii*. Thus, we might wonder why the *A. mertensii*–*P. goeldii* association persists. An evolutionary response that might eliminate the *P. goeldii*-associated plants over evolutionary time can only occur if variations in the traits related to the mutualism are heritable; specifically, variation must exist in traits that might determine whether a plant is associated with one or the other ant species. Studies have demonstrated that at least some AG-plants are equipped with aromatic compounds, perhaps genetically determined, that might make their seeds more or less attractive to ants (Seidel *et al.*, 1990), and potentially differentially attractive to different ant species. If there is genetic variation in plant traits that influence the identity of the mutualistic ant, several hypotheses might explain the continued persistence of the *P. goeldii* association. First, trade-offs may exist between resource acquisition and inbreeding, with *C. femoratus*-associated plants acquiring more resources but also suffering more inbreeding than *P. goeldii*-associated plants. *Pachycondyla goeldii* workers, which very rarely leave their nest during the daytime, might be less aggressive towards pollinators than *C. femoratus* workers that – day and night – may instantaneously react to any motion or disturbance of the AG. In this way, *P. goeldii*-associated plants might experience an outcrossing advantage through the ant mutualist's impact on pollinators. Second, the fruits and seeds of *P. goeldii*-associated bromeliads are dispersed by insects/animals whereas those associated with *C. femoratus* are, in part, harvested by the ants which then disperse the seeds in their own AG (J. Orivel, UMR Ecologie des Forêts de Guyane, French

Guiana, pers. comm.). If the seeds of *P. goeldii*-associated bromeliads are dispersed further away than those from *C. femoratus*-associated bromeliads, *P. goeldii*-associated plants may benefit from advantages related to enhanced seed dispersal (Howe and Smallwood, 1982). Third, traits that might specialize plants to one mutualist (*C. femoratus*) may themselves be selected against compared with traits that permit more generalization in mutualist identity because specialization can reduce the assurance that the seeds become part of any AG (an argument analogous to the concept in pollination biology that generalist pollination systems are favoured due to reproductive assurance; Waser *et al.*, 1996). *Aechmea mertensii* seed dispersal, even by an apparently less favourable ant species, is important because this bromeliad species occurs exclusively in association with arboreal ants, and has never been found growing outside AGs (Madison, 1979; Benzing, 2000).

Divergence in flower size and shape among a plant population is largely explained on the basis of pollinator- or florivore-mediated selection. In the present study, we provide evidence for the first time of the importance of the identity of the mutualistic ants on inflorescence, floral and fruit traits. The strength and direction of this selection on floral and fruit traits change depending on the ant species, which may play a contrasting role in shaping plant evolution and speciation. However, as the reproductive biology of *A. mertensii* is still very poorly known, further experiments and studies are needed to better understand its breeding system and the mechanisms of microevolution such as gene flow, as well as genetic drift and selection in the context of ant–plant interactions.

ACKNOWLEDGEMENTS

We are grateful to the members of the 'Laboratoire Hydréco de Petit-Saut' for their logistical help, to Olivier Roux for his help in the field and to Andrea Yockey-Dejean for proofreading the manuscript. We would also like to thank the three anonymous reviewers for valuable comments on previous versions of the manuscript. Financial support for this study was provided through the Programme Amazonie II of the French Centre National de la Recherche Scientifique (Project 2ID), and the Programme Convergence 2007–2013 (Région Guyane) from the European Community (Project DEGA).

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